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PDGF and TGF-α Act Synergistically to Improve Wound Healing in the Genetically Diabetic Mouse

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Impaired wound healing results in significant morbidity for the surgical patient. The genetically diabetic (C57BL/KsJ-db/db) mouse is obese, hyperglycemic, insulin-resistant, and exhibits markedly impaired wound healing. Previous studies have demonstrated that the fibroblast mitogens, BB homodimer of platelet-derived growth factor (PDGF-BB) or basic fibroblast growth factor, plus insulin-like growth factor, act synergistically to enhance wound closure in the genetically diabetic mouse. The purpose of this study was to determine whether the keratinocyte mitogens, epidermal growth factor (EGF) or transforming growth factor-α (TGF-α), in combination with the fibroblast mitogen, PDGF-BB, would produce a similar synergistic enhancement in tissue repair. Full-thickness skin wounds created on the backs of diabetic mice received topical applications of vehicle (5% polyethylene glycol), PDGF-BB (10 μg), EGF (1 μg), TGF-α (1 μg), or the combination of PDGF (10 μg) and EGF (1 μg) or TGF-α (1 μg) for 5 consecutive days starting at wounding. Application of PDGF-BB or TGF-α alone to wounds in diabetic animals improved wound closure when compared to vehicle treatment. EGF did not affect healing and did not have any additive effects when combined with PDGF-BB. Significant improvements in wound closure were observed with the combination of PDGF-BB and TGF-α when compared to treatment with the individual growth factors. The PDGF-BB/TGF-α combination accelerated healing in the diabetic animals to a rate that was closer to that seen in nondiabetic mice. By histologic analysis at Day 15, all criteria for healing were more advanced in the PDGF-BB/TGF-α combination wounds when compared to the other treatment groups. As all wounds approached complete healing by Day 21, these differences were lost. In summary, PDGF-BB and TGF-α acted synergistically in genetically diabetic mice to promote early wound healing beyond that of the individual growth factors. Similar synergy was not observed with the combination of PDGF-BB and EGF. © 1994 Academic Press, Inc.

INTRODUCTION

Impaired wound healing is a significant source of morbidity for the surgical patient and may result in such complications as wound dehiscence, anastomotic breakdown, and chronic nonhealing wounds. The cost associated with such complications may be extreme due to prolonged hospitalization and increased time away from work. In the normal host, wound healing is usually uncomplicated and proceeds at a rapid rate. In contrast, most healing failures are associated with some form of host impairment, including diabetes, infection, immunosuppression, obesity, or malnutrition [1–4]. For greater clinical relevance, wound healing studies should focus on models of impaired healing.

Wound healing is a complex biologic process which may be divided into three phases—inflammatory, proliferative, and maturational. Growth factors appear to play an important role in all phases of this process by orchestrating complex cell-to-cell interactions which are essential for successful wound repair. Growth factors, which are released into the wound site at the time of injury, have been shown to regulate cellular migration [5–8] and proliferation [9–12] as well as extracellular matrix deposition and remodeling [13–17]. Multiple growth factors or their transcripts have been identified in healing wounds, including platelet-derived growth factor (PDGF) [18–23], basic fibroblast growth factor (bFGF) [20], epidermal growth factor (EGF) [20, 24], transforming growth factor-α (TGF-α) [18, 25, 26], transforming growth factor-β (TGF-β) [18, 26, 27], insulin-like growth factor-I (IGF-I) [18, 28], and insulin-like growth factor-II (IGF-II) [28]. The specific interactions of these growth factors, however, are not well understood.

It has been hypothesized that impaired wound healing may result from lack of adequate stimulation by growth factors. Schultz *et al.* [29] report that fluid obtained from

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chest drains of healing mastectomy wounds contained high levels of growth factors and stimulated high levels of DNA synthesis by fibroblast cultures. In contrast, fluids collected from chronic, nonhealing wounds had low levels of growth factors and failed to stimulate DNA synthesis. Indeed, growth factors have been shown to improve wound repair in animals with diabetes [30–36], infection [37], malnutrition [38], or in those impaired as a result of steroids [39–41], chemotherapeutic agents [42, 43], or radiation [44]. Clinical trials investigating the use of growth factors to improve wound closure in patients with chronic, nonhealing wounds [45–51] or partial-thickness donor sites [52, 53] have also been performed.

Although significant enhancement of wound closure may be obtained with single growth factors, several studies have demonstrated a more pronounced effect using combinations of growth factors, suggesting synergy [54–58]. It is logical that combinations of growth factors would be more effective than individual growth factors since, in the wound, multiple growth factors are present and presumably active.

Previous studies from this laboratory have demonstrated that the fibroblast mitogens, PDGF-BB or bFGF, act synergistically with IGF-II to improve wound healing in the genetically diabetic mouse [36, 59]. The most profound improvement in healing was achieved with the combination of PDGF-BB and IGF-II [36]. Lynch *et al.* reported similar findings with the combination of PDGF-BB and IGF-I in partial-thickness porcine skin wounds [54, 55] and canine bone regeneration models [56, 57].

Although functions may overlap, fibroblast mitogens act primarily to stimulate fibroblast replication, extracellular matrix deposition, and remodeling, while keratinocyte mitogens predominantly affect epithelial proliferation and migration. The purpose of this study was to determine whether the combination of a keratinocyte mitogen, EGF or TGF-α, and a fibroblast mitogen, PDGF-BB, would produce a synergistic effect similar to that previously seen with the fibroblast mitogens in the genetically diabetic mouse.

METHODS AND MATERIALS

Animals

Female genetically diabetic (C57BL/KsJ-db/db) mice, ages 8–12 weeks and weighing 35–45 g, were obtained from Jackson Laboratories (Bar Harbor, ME). During experiments, animals were housed in individual cages in a central animal care facility, maintain d on a 12-h light–dark cycle, and given free access to standard rodent chow and water. The animal care facilities were maintained by professionals in accordanc with federal guidelines, and all procedur s were approved by the University of Cincinnati Institutional Animal Care Utilization Committee.

Tissue repair in the genetically diabetic (C57BL/KsJ-db/db) mouse has been proven to be a clinically relevant model of impaired wound healing. The animals exhibit several characteristics of human adult-onset diabetes including obesity, insulin-resistant hyperglycemia, and markedly delayed wound closure. These abnormalities arise as a result of a single autosomal recessive mutation on chromosome 4 [60]. In our laboratory, serum glucos levels in the diabetic mice averaged greater than 900 mg/dl with insulin levels more than doubling levels found in the nondiabetic littermates [34]. The diabetic animals were not treated with insulin because of their marked resistance and because the goal was to examine healing in diabetes. Only the homozygous carriers of the recessive gene develop signs of diabetes. Heterozygotes are clinically unaffected, and wound healing proceeds at a normal rate. Because previous studies from this laboratory have documented that growth factors have no effect on enhancement of wound closure in the heterozygous nondiabetic littermate, this study was performed in homozygous diabetic animals. A representative healing curve of nondiabetic heterozygotes has been included for comparison purposes.

Wounding

The animals were anesthetized using methoxyflurane inhalation (Metofane; Pitman–Moore, Inc., Mundelein, IL). After clipping the hair on the back, the skin was prepped with povidone–iodine solution and wiped with 30% isopropyl alcohol. Using a sterile template, a full-thickness wound measuring 1.5 × 1.5 cm was created by excising the skin on the mid-back, including the panniculus carnosus. Tincture of benzoin (Compound Benzoin Tincture U.S.P.; Cumberland-Swan, Inc., Smyrna, TN) was applied to the perimeter of the wound and allowed to dry. The wound was then covered with a transparent, semipermeable polyurethane dressing (OpSite; Smith and Nephew Medical Limited, Hull, England) and sealed at the edges by the benzoin. The transparent OpSite dressing allowed for easy visualization of the wound.

Growth Factors

Human recombinant PDGF-BB was generously provided by Zymogenetics, Inc. (Seattle, WA). Recombinant human TGF-α was purchased from R & D Systems, Inc. (Minneapolis, MN). Recombinant human EGF was purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Growth factors were mixed in a vehicle of sterilely filtered 5% polyethylene glycol (average mw 8000; Aldrich Chemical Company, Inc., Milwaukee, WI).

Animals were randomly assigned to treatment groups, and investigators were blinded until completion of all observations. The dose for ach mouse was prepared

prior to wounding and stored in individual syringes at 4°C. Approximately 0.6 ml of treatment solution was prepared for each mouse, and 0.1 ml of the solution was applied once daily for 5 consecutive days starting at wounding by injecting it beneath the OpSite dressing and allowing it to spread over the wound. Wounds received topical application of vehicle (5% polyethylene glycol), PDGF-BB (10 μg/dose), TGF-α (1 μg/dose), EGF (1 μg/dose), or the combination of PDGF-BB (10 μg/dose) and TGF-α (1 μg/dose) or EGF (1 μg/dose). Treatment groups consisted of 8 to 10 animals per group, and experiments were repeated two to three times to document consistency of results. Because of the variability of healing for each group of animals, each experiment included its own vehicle control.

Wound Analysis

Diabetic animals heal their wounds by granulation tissue formation and reepithelialization rather than by contraction [34, 36]. The edge of migrating epithelium is easily discernible from the moist granulation tissue. Histology confirms the presence of the epithelial border as the edge of the healing wound.

The edges of epithelial migration were serially traced onto glass slides on days 0, 3, 7, 11, 15, 18, and 21. The areas of the traced wounds were measured by planimetry using an image analysis system (Image-1; Universal Imaging Corporation, Media, PA), and percentage wound closure was determined. Wound closure is expressed as percentage closure of the original wound and is calculated as

$$\%\ \text{wound closure} = [(\text{Day 0 area} - \text{Day } N \text{ area})/\text{Day 0 area}] \times 100.$$

After the final wound tracing on either Day 15 or Day 21, the animals were given a lethal intraperitoneal injection of pentobarbital, and the entire wound, including a 5-mm margin of unwounded skin, was excised down to the fascia. The wound was divided in half through the least healed portion. One-half of the wound was placed in 10% formalin (Forma-Scent Fixative; Baxter Healthcare Corporation, McGraw Park, IL) for histologic analysis, and the other half was placed in liquid nitrogen and stored at −70°C for future molecular studies. Wound sections were stained with hematoxylin and eosin, and Masson's trichrome. Each wound section was evaluated by two blinded investigators and assigned a histologic score ranging from 1 (no healing) to 12 (complete healing) based on degree of cellular infiltration, granulation tissue formation, vascularity, and reepithelialization (Table 1). The complete histologic scoring system has been previously published [34].

Data Analysis

Values are expressed as means ± standard error of the mean (SEM). Comparisons of the extent of wound closure were made with one-between, one-within, repeated measures analysis of variance (RM-ANOVA), with Duncan's multiple range test being used for individual comparisons. Statistical analysis of the histologic scores was performed using the Wilcoxon rank-sum test. Values with $P < 0.05$ were considered significant. Data analysis was performed with SAS software (version 6.04; SAS Institute, Inc., Cary, NC).

TABLE 1

Histology Scoring Syst m

Score	Criteria
1–3	None to minimal cell accumulation. No granulation tissue or epithelial travel.
4–6	Thin, immature granulation that is dominated by inflammatory cells but has few fibroblasts, capillaries, or collagen deposition. Minimal epithelial migration.
7–9	Moderately thick granulation tissue, can range from being dominated by inflammatory cells to more fibroblasts and collagen deposition. Extensive neovascularization. Epithelium can range from minimal to moderate migration.
10–12	Thick, vascular granulation tissue dominated by fibroblasts and extensive collagen deposition. Epithelium partially to completely covering the wound.

Note. From Ref. 34 with permission of the publisher.

RESULTS

Genetically diabetic animals tolerated the wounding procedures well. An occasional wound (approximately 3%) developed frank purulence and was eliminated from the data analysis because of probable infection. The rest of the animals tended to gain weight and showed no overt signs of illness.

In the first experiment, the effects of combining of PDGF-BB (10 μg) and EGF (1 μg) were studied (Fig. 1, $N = 8$/group). As previously documented, healing in the vehicle-treated diabetic animals was significantly delayed when compared with the typical healing of nondiabetic mice. Since the purpose was to compare the effects of growth factors in diabetic animals, nondiabetics were not included in the experiments. A representative healing curve of a group of untreated nondiabetic animals ($N = 12$) is included for comparison but was not included in the statistical analysis. Treatment of wounds in diabetic animals with PDGF-BB enhanced wound closure when compared to vehicle treatment. The keratinocyte mitogen EGF did not have any effect on healing. Furthermore, EGF was unable to further enhance the beneficial effects of PDGF-BB. Histologic analysis of Day 21 wounds support these findings. The high st histologic score was seen with PDGF-BB (10.25 ± 0.63), followed

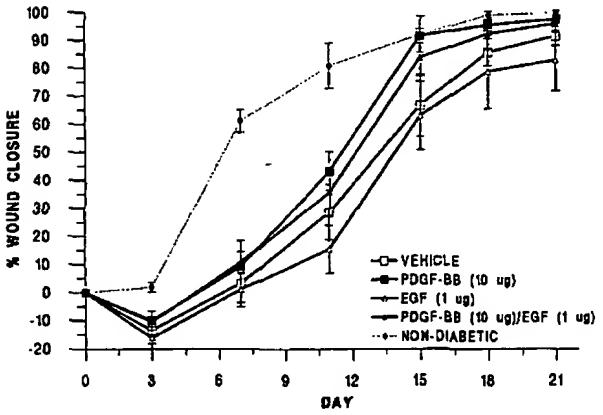

FIG. 1. Percentage wound closure (means ± SEM) in genetically diabetic (C57BL/KsJ-db/db) mice (N = 8/group) treated with vehicle (5% polyethylene glycol), PDGF-BB (10 μg), EGF (1 μg), or the combination of PDGF-BB (10 μg) and EGF (1 μg). There are no significant differences between groups by RM-ANOVA. A representative nondiabetic healing curve is included for comparison purposes but was not included in the statistical analysis.

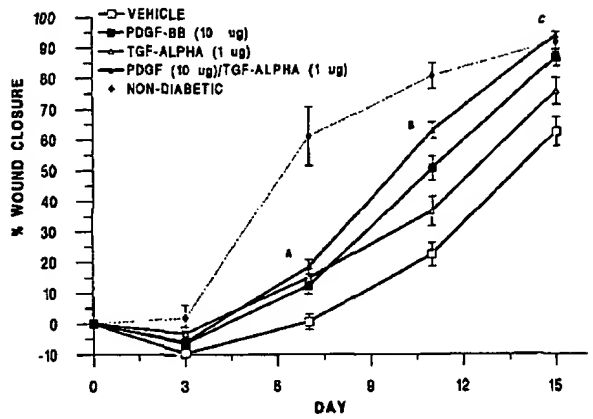

FIG. 3. Percentage wound closure (means ± SEM) in genetically diabetic (C57BL/KsJ-db/db) mice treated with vehicle (5% polyethylene glycol), PDGF-BB (10 μg), TGF-α (1 μg), or the combination of PDGF-BB (10 μg) and TGF-α (1 μg). Cumulative data from four experiments (N = 36/group) reveals that the combination of PDGF-BB and TGF-α consistently enhances healing beyond that seen with PDGF-BB or TGF-α alone. (A) $P < 0.05$ for PDGF-BB, TGF-α, or PDGF-BB/TGF-α vs vehicle; (B) all groups are significantly ($P < 0.05$) different from each other; and (C) $P < 0.05$ for PDGF-BB, TGF-α, or PDGF-BB/TGF-α vs vehicle and PDGF-BB/TGF-α vs TGF-α.

by the combination of PDGF-BB and EGF (9.38 ± 0.81), vehicle (8.88 ± 1.10), and EGF (7.75 ± 0.85).

Since TGF-α is a more potent keratinocyte mitogen than EGF, the next experiment was designed to determine whether the combination of PDGF-BB (10 μg) and TGF-α (1 μg) would have the synergistic effect that was lacking for PDGF-BB (10 μg) and EGF (1 μg). Vehicle-treated diabetic wounds continued to have the greatest delay in healing (Fig. 2, N = 10/group). (The same nondiabetic healing curve is included for comparison and these animals were not included in the statistical analysis.) Topical PDGF-BB alone significantly improved healing when compared to vehicle. TGF-α tended to have greater wound closure than vehicle treatment, but the effect was not significant. Statistically significant ($P < 0.05$) improvements in wound closure were observed after treatment with the PDGF-BB/TGF-α combination on Day 7 versus vehicle, on Day 11 versus vehicle, TGF-α, and PDGF-BB, on Day 15 versus vehicle and TGF-α, and on Day 18 versus vehicle. By 21 days all of the wounds had approached complete healing so no differences were noted on that day. The significant augmentation in healing observed with the PDGF-BB/TGF-α combination when compared to healing observed after treatment with either PDGF-BB or TGF-α alone indicates that the two growth factors have at least some synergistic effects. The enhancement seen after treatment with both PDGF-BB and TGF-α approached the healing seen in nondiabetic mice.

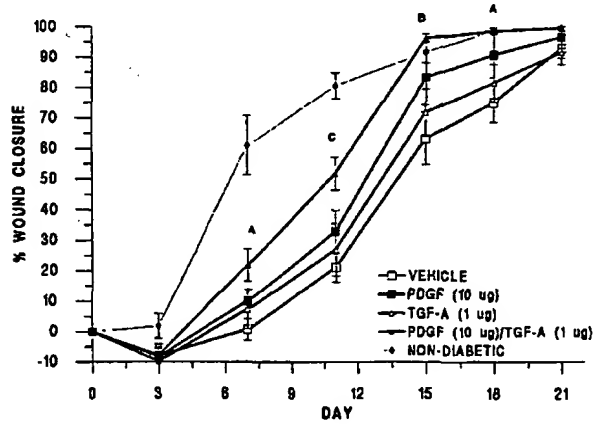

FIG. 2. Percentage wound closure (means ± SEM) in genetically diabetic (C57BL/KsJ-db/db) mice (N = 10/group) treated with vehicle (5% polyethylene glycol), PDGF-BB (10 μg), TGF-α (1 μg), or the combination of PDGF (10 μg) and TGF-α (1 μg). The same nondiabetic healing curve is included for comparison. (A) $P < 0.05$ vs vehicle; (B) $P < 0.05$ vs vehicle and TGF-α; and (C) $P < 0.05$ vs vehicle, TGF-α, and PDGF-BB by ANOVA and Duncan's multiple range test.

The synergistic effects of the PDGF-BB/TGF-α combination were confirmed by repeating experiments three times. In two studies, the wounds were followed for only 15 days to examine differences in histology. The pooled data adds further evidence to the synergistic effects of PDGF-BB and TGF-α (Fig. 3, N = 36/group). By Day 7, all of the growth factor treatment groups had significantly greater wound closure than vehicle. At Day 11, significant differences existed for all of the groups. In other words, PDGF-BB/TGF-α > PDGF-BB > TGF-α > vehicle. On Day 15, the PDGF-BB/TGF-α combination treatment was significantly better than TGF-α, and all growth factor treatments healed to a greater extent than those wounds treated with vehicle.

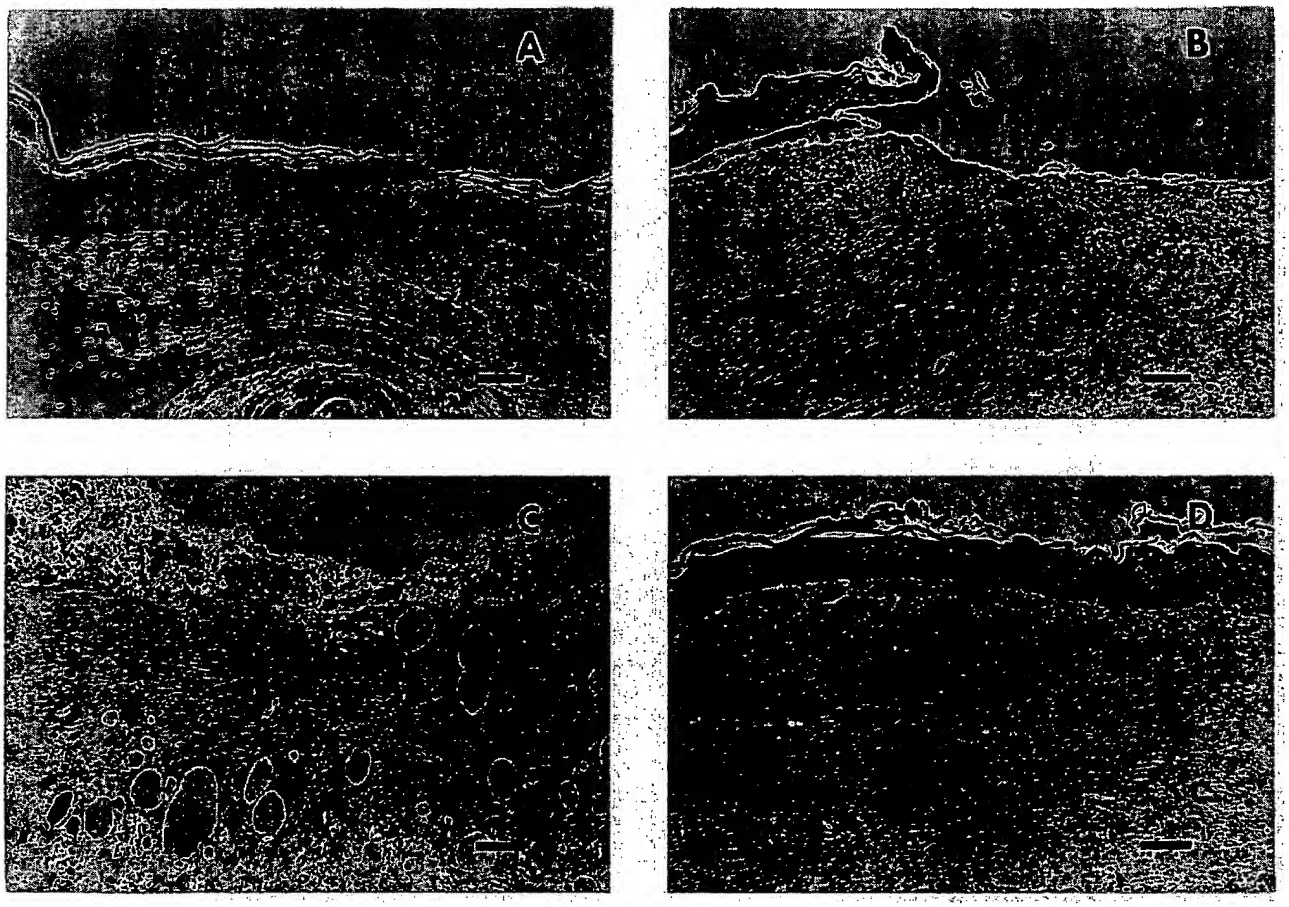

FIG. 4. Hematoxylin and eosin-stained sections of Day 15 wounds. (A) Vehicle; (B) PDGF-BB; (C) TGF-α; (D) PDGF-BB and TGF-α (bar = 100 μm). Vehicle-treated wounds reveal a paucity of cellular entry by Day 15. Granulation tissue is thin and immature, and epithelial travel is minimal. Although they exhibit different *in vitro* activities, the addition of either PDGF-BB or TGF-α results in increased cellular entry and more mature granulation tissue, but little epithelial migration. The combination of PDGF-BB and TGF-α, however, results in formation of thick, vascular, mature granulation tissue and complete reepithelialization of the wound.

Histologic analysis of Day 15 wounds supports the improved wound closure observed with the combination of PDGF-BB and TGF-α. Representative hematoxylin and eosin-stained histologic sections are shown in Fig. 4. Wounds in diabetic animals treated with vehicle had a paucity of cellular entry by Day 15. The limited granulation tissue was thin, dominated by inflammatory cells, and minimally covered by epithelium. Although exhibiting different *in vitro* activities, the addition of either PDGF-BB or TGF-α resulted in similar increases in cellularity that contained a mix of inflammatory cells and fibroblasts. Epithelial migration was limited at Day 15. The combination of PDGF-BB and TGF-α resulted in formation of thick, vascular, mature granulation tissue that tended to be populated with less inflammatory cells and more fibroblasts. Trichrome-stained histologic sections at Day 15 (not shown) revealed increased collagen deposition in wounds treated with the combination of PDGF-BB and TGF-α when compared to the other treatment groups. Epithelial coverage was always greater in those wounds treated with the PDGF-BB/TGF-α combination. The decrease in inflammatory cells along with the increase in fibroblasts and collagen suggest that the wounds have reached a greater degree of maturation. As wounds mature, inflammation resolves and collagen deposition dominates. Histologic evaluation also suggests that an adequate granulating bed is required prior to epithelial migration in these wounds. The histologic scores for the various groups at Days 15 and 21 confirm the above observations (Table 2).

DISCUSSION

The genetically diabetic (C57BL/KsJ-db/db) mouse is a clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and reepithelialization rather than contraction [34, 36]. The de-

TABLE 2

Histologic Scores (Values Expressed as Means ± SEM)

Treatment	Day 15	Day 21
Vehicle	7.38 ± 0.58 (13)	8.73 ± 0.45 (13)
PDGF-BB (10 μg)	8.25 ± 0.41 (12)	9.85 ± 0.52 (13)
TGF-α (1 μg)	6.88 ± 0.66 (12)	9.46 ± 0.62 (12)
PDGF-BB (10 μg) and TGF-α (1 μg)	9.54 ± 0.55 (12)*	10.15 ± 0.49 (13)

* $P < 0.05$ vs vehicle and TGF-α; $P = 0.06$ vs PDGF-BB by Wilcoxon rank-sum test.

fect in tissue repair has been related to a delay in cellular entry which makes this an attractive model for study of growth factor effects. Once inflammatory cells arrive in the wound, healing appears to proceed relatively normally. Exogenous application of growth factors appears to draw inflammatory cells into the wound and accelerate granulation tissue formation. It appears that epithelial migration depends on the deposition of an adequate granulation bed. The dependence on granulation tissue is supported by the findings that EGF has no effect in accelerating healing in these animals. TGF-α has been shown to enhance fibroblast proliferation and have an angiogenic effect, in addition to its ability to enhance epithelial migration and proliferation [61]. The moderate ability of TGF-α to enhance granulation tissue formation in the diabetic animals could result from these effects.

PDGF is an approximately 30,000 MW polypeptide growth factor which is a disulfide-linked dimer consisting of two chains—A and B. It exists in three isomeric forms—PDGF-AB, PDGF-AA, and PDGF-BB. The PDGF-BB isomer was utilized in all of our experiments, but similar enhancement has been found following application of PDGF-AA [62]. PDGF is released from α-granules of platelets at the time of injury and by activated macrophages later in the wound healing process. PDGF may also be released from smooth muscle cells and endothelial cells. It is a potent mitogen for cells of mesenchymal origin, including fibroblasts and smooth muscle cells. Likewise, PDGF is a potent chemotactic agent for fibroblasts, smooth muscle cells, monocytes, and neutrophils. It serves an important role in modification of the extracellular matrix by stimulating collagen, collagenase, and glycosaminoglycan synthesis [63]. PDGF may also play a role in angiogenesis, which may be either indirect by stimulating macrophages to produce angiogenic factors or a direct action on vascular endothelial cells [64].

In vivo, PDGF has been shown to significantly enhance tissue repair in several wounding models [30, 34, 40, 44, 65–69]. In our study, the application of PDGF-BB to full-thickness diabetic mouse wounds improved wound closure beyond that of vehicle, EGF, or TGF-α and resulted in more mature granulation tissue with increased collagen deposition.

Keratinocyte mitogens also play an important rol in the wound healing process. The most thoroughly studied keratinocyte mitogens are EGF and TGF-α, which belong to the same family and have similar mechanisms of action [29]. Both EGF and TGF-α are approximately 6000 MW single-chain polypeptides which share about 40% amino acid homology. The biologic activities of both EGF and TGF-α are mediated through the EGF receptor. Both are potent keratinocyte mitogens which stimulate both epithelial proliferation and migration. Additionally, both growth factors are mitogenic for fibroblasts and endothelial cells. Studies suggest that TGF-α is more potent than EGF *in vivo* both as an angiogenic factor [61] and as a promoter of epidermal r - generation [70].

EGF has been shown to enhance wound healing in several animal models [32, 68, 71–74] and has produced equivocal results in clinical trials in patients with partial-thickness donor sites [45] and chronic wounds [48]. Lynch *et al.* [55] reported no significant improvement in healing with the application of TGF-α to partial-thickness porcine skin wounds, but did find a significant synergistic effect with the combination of PDGF and TGF-α. In our study, topical application of EGF to full-thickness diabetic mouse wounds resulted in no significant improvement in wound healing above that of vehicl . Histologic analysis of EGF-treated wounds revealed relatively immature granulation tissue and minimal to no epithelial travel. On the other hand, treatment with TGF-α resulted in improved wound closure above that of vehicle, characterized histologically by thicker, mor mature granulation tissue, but still with little epithelial travel. Increased vascularity was also observed in wounds treated with TGF-α. These results suggest that TGF-α is a more potent fibroblast mitogen and angiogenic factor than EGF. Although not tested, higher doses of EGF may have produced an enhancement of wound closure in the diabetic animals. PDGF, however, appears to the most active of the three. These findings lend further support to the hypothesis that formation of a thick, vascular granulation tissue base is required for reepithelialization to proceed. One explanation for the stimulatory effects of TGF-α and not EGF could be that the former had more pronounced effects on fibroblasts and angiogenesis than EGF. The stimulation of keratinocyte migration and proliferation, a dominant effect of EGF, might be ineffective in the lack of an adequate wound bed.

Several studies have documented the synergistic effects of growth factor combinations *in vitro* [75–78]. Stiles *et al.* [75] d monstrated the requirement for both a "competence" factor (i.e., PDGF or FGF) and a "progression" factor (i.e., IGF-I or IGF-II) for progression of BALB/c 3T3 cells into the S phase of the cell cycle. *In vivo* studies in this laboratory have docum nted the syn-

ergistic effects of the combination of PDGF-BB or bFGF (competence factors) with IGF-II (progression factor) on enhancing wound repair in the genetically diabetic mouse. Lynch *et al.* likewise demonstrated synergism with PDGF and IGF-I in partial-thickness porcine skin wounds [54, 55] and canine bone regeneration models [56, 56].

The purpose of the present study was to determine whether the combination of a potent fibroblast mitogen, PDGF-BB, with a keratinocyte mitogen, EGF or TGF-α, would act synergistically to improve wound healing in the genetically diabetic mouse. The results demonstrated that no synergistic effect was obtained with the combination of PDGF-BB and EGF. In contrast, PDGF-BB and TGF-α appear to act synergistically to promote wound healing beyond that of the individual growth factors. As previously discussed, Lynch *et al.* [55] reported similar synergy with the combination of PDGF and TGF-α for treatment of partial-thickness porcine skin wounds.

Further studies should investigate the effects of the combination of PDGF-BB (fibroblast competence factor), IGF-II (fibroblast progression factor), and TGF-α (keratinocyte mitogen) to determine if a more pronounced synergistic effect could be achieved. Likewise, the effects of a different fibroblast mitogen (i.e., bFGF) in combination with TGF-α should be studied.

Although these *in vivo* studies provide insight into possible mechanisms of wound repair in the impaired host, many questions will remain unanswered and will remain until the complex interactions of growth factors at the molecular level are unraveled. It does appear, however, that growth factor combinations may eventually become a valuable adjuvant for the clinical management of chronic wounds, including diabetic ulcers, venous stasis ulcers, and major burns.

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